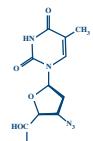


## Toxicology and Carcinogenesis Studies of Transplacental AZT

## Swiss (CD-1) Mice

## In Utero Exposure Studies

TR 522

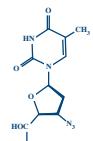


3'-AZIDO-3'-DEOXYTHYMIDINE  
CAS No. 30516-87-1

MW: 267.24

## Zidovudine

**AZT - Drug used to treat HIV infections and prevent mother-to-child transmission of HIV**

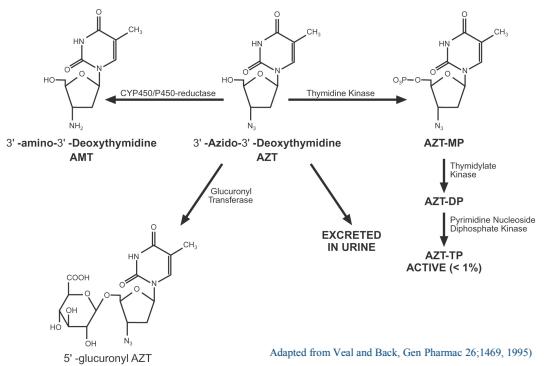


3'-AZIDO-3'-DEOXYTHYMIDINE  
CAS No. 30516-87-1

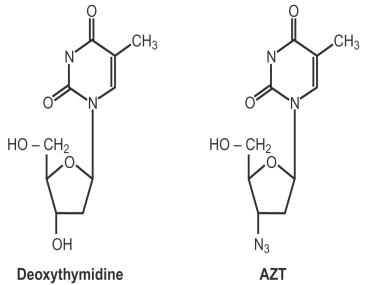
MW: 267.24

## Zidovudine

AZT Metabolism



Adapted from Veal and Back, Gen Pharmac 26;1469, 1995



AZT-TP inhibits thymidine-TP incorporation into DNA  
(3' hydroxyl group replaced with azido [N<sub>3</sub>] group)

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### **AZT - DNA chain terminator**

- ◆ AZT-TP has selective affinity for HIV reverse transcriptase
- ◆ Host cell DNA polymerases 50-100-fold less sensitive to AZT than HIV reverse transcriptase
- ◆ Mitochondrial DNA polymerase gamma is inhibited by AZT-TP

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### **AZT - genotoxic**

- ◆ Salmonella positive - with/without S9
- ◆ CHO cells - positive for sister chromatid exchange
- ◆ Micronucleated erythrocytes in mouse bone marrow/peripheral blood
- ◆ Mitochondria damage - humans, rodents, primates

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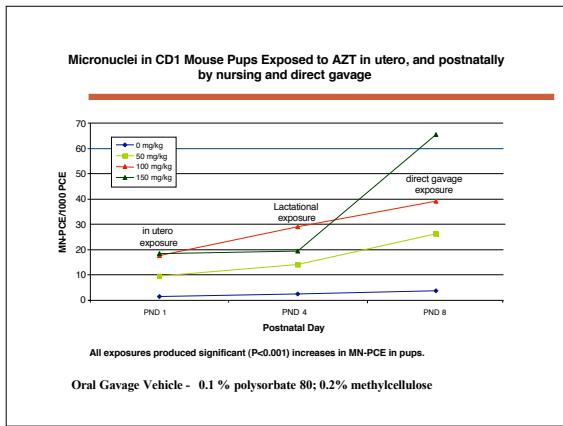
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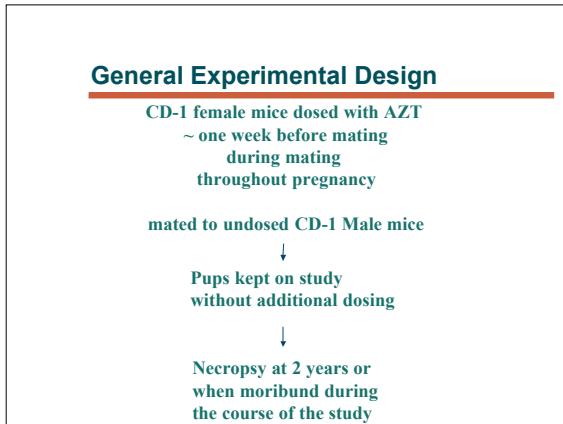
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**AZT Study Background**

- NIEHS studies designed to determine cancer effects in CD-1 pups after dams were given AZT at 50 - 300 mg/kg/day throughout gestation



### AZT - Dosing of dams

- ◆ Total daily doses: 0, 50, 100, 200, or 300 mg AZT/kg/day
- ◆ Daily doses divided in two equal doses 6 hours apart
- ◆ 10mL/kg/dose for a total volume of 20 mL/kg/day
- ◆ Vehicle - 0.5% methylcellulose

### AZT Exposure levels

	mg/kg	mg/m <sup>2</sup>
Human Dose	8	296
Mouse Dose	50	150
	100	300
	200	600
	300	900

mg/m<sup>2</sup> = K<sub>m</sub> x (dose in mg/kg); K<sub>m-human</sub> =37; K<sub>m-mouse</sub> = 3  
Freireich et al, 1966

### Pups exposed in utero - 0, 50, 100 mg AZT/kg/day

- ◆ Dose groups 0, 50, 100 mg AZT/kg/day - pups culled on postnatal day 4 to 4-5 pups/sex/litter
- ◆ Up to 4 pups/sex/litter kept on study without additional dosing for two years

### **Pups exposed in utero - 200 and 300 mg/kg/day**

- ◆ Dose groups 200 or 300 mg AZT/kg/day - no pups culled because of reduced litter size
- ◆ All surviving pups kept on study without additional dosing for two years

### **Results of Mating**

AZT Dose groups mg/kg/day	0	50	100	200	300
Number of Dams in group	22	22	28	34	46
% of dams pregnant	82%	95%	68%	56%*	39%**

\* p <0.05 \*\* p<0.01

### **Average no. of pups per litter - postnatal day 0**

AZT Dose groups mg/kg/day	0	50	100	200	300
Average Pups per litter	11	9.2	8.2	4.4**	4.2**

\*\* p < 0.01

### No. of Mice per group

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AZT mg/kg/day	0	50	100	200	300
Males Start	50	50	50	37	32
Females Start	50	50	50	40	42

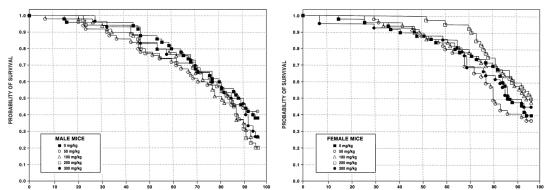
### Survival at Study termination

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AZT mg/kg/day	0	50	100	200	300
Males	19	21	13	7	8
%Survival	38%	42%	26%	19%	36%
Females	21	21	14	8	10
% Survival	42%	42%	28%	20%	24%

### AZT Pup Survival Curves

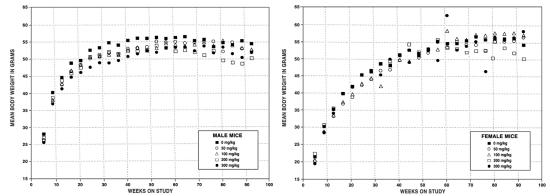
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### Final Mean Body weights - % of controls

AZT Dose groups mg/kg/day	0	50	100	200	300
Male Mice	-	96%	97%	92%	95%
Female Mice	-	104%	105%	92%	107%

### AZT Pup Growth Curve



### Male Mice - Lung Alveolar/Bronchiolar Adenoma

AZT mg/kg/day	0	50	100	200	300
Adenoma	10/50	13/50	8/50	9/37	11/32
Incidence %	20%	26%	16%	24%	34%
Poly-3-test	0.109	0.311	0.480N	0.361	0.137

### Male Mice - Lung Alveolar/Bronchiolar Carcinoma

AZT mg/kg/day	0	50	100	200	300
Carcinoma	5/50**	11/50	6/50	11/37**	8/32*
Incidence %	10%	22%	12%	30%	25%
Poly-3-test	0.014*a	0.068	0.422	0.009**	0.043*

\*p<0.05 \*\*p<0.01 \*a trend statistic

### Male Mice - Lung Alveolar/Bronchiolar Adenoma/Carcinoma

AZT mg/kg/day	0	50	100	200	300
Adenoma or carcinoma	14/50	20/50	13/50	18/37*	18/32**
Incidence %	28%	40%	26%	49%	56%
Poly-3-test	0.002***	0.156	0.592	0.027*	0.007**

\*p<0.05 \*\*p<0.01 \*a trend statistic

### Female Mice - Lung Alveolar/Bronchiolar Adenoma/Carcinoma

AZT mg/kg/day	0	50	100	200	300
Adenoma or carcinoma	10/50	11/49	14/50	12/40	11/42
Incidence %	20%	22%	28%	30%	26%
Poly-3-test	0.38	0.32	0.40	0.38	0.39

## Conclusion

- ♦ **Some evidence** of carcinogenic activity in **F<sub>1</sub> male mice** exposed transplacentally to AZT
  - Increased incidence of alveolar/bronchiolar neoplasms
- ♦ **No evidence** of carcinogenic activity in **F<sub>1</sub> female mice** exposed transplacentally to AZT at 50, 100, 200, or 300 mg/kg
- ♦ **Decreased litter size and fertility rates** in dams at 200 and 300 mg/kg

## NIEHS Molecular Biology Studies

L. Hong and R. Sills  
Laboratory of Experimental Pathology  
Preliminary Results



### Pattern of K-ras Mutations in Lung Neoplasms from Swiss (CD-1) Male Mice in the AZT Transplacental Carcinogenesis Study

Treatment	Activated K-ras (%)	Codon 12 (Normal-GGT)					Codon 13 (Normal-GGC)	
		GAT	TGT	GTT	CGT	AGT	AGT	AGC
Concurrent Control <sup>a</sup>	0/2 (0 %) 25/38 (66%)	0	0	0	0	0	0	0
AZT <sup>a</sup>		4	18	0	1	1	1	0
Alveolar/Bronchiolar								
Adenoma	5/11 (45%)	1	4	0	0	0	0	0
Carcinoma	20/27 (74%)	3	14	0	1	1	1	0
AZT 50mg/kg	7/11 (64%)	2	4	0	1	0	0	0
100mg/kg	4/5 (80%)	0	4	0	0	0	0	0
200mg/kg	6/11 (55%)	1	3	0	0	1	1	0
300mg/kg	8/11 (73%)	1	7	0	0	0	0	0

<sup>a</sup>Male Swiss (CD-1) mice were exposed to 0, 50, 100, 200, or 300 mg/kg AZT

Predominant K-ras Mutations in Lung Neoplasms from Swiss (CD-1)  
Male Mice in the AZT Transplacental Carcinogenesis Study

Exon 1  
Codon 12  
~~G~~GT → TGT  
Gly → Cys

Pattern of p53 Mutations in Lung Neoplasms from Swiss (CD-1)  
Male Mice in the AZT Transplacental Carcinogenesis Study

Treatment	P53 Mutations (%)	Exon 5	Exon 8
Concurrent Control*	0/2 (0 %)	0	0
AZT*	32/38 (84%)	16	16
Alveolar/Bronchiolar			
Adenoma	9/11 (82%)	4	5
Carcinoma	23/27 (85%)	12	11
AZT 50mg/kg	8/11 (73%)	4	4
100mg/kg	5/5 (100%)	2	3
200mg/kg	11/11 (100%)	6	5
300mg/kg	8/11 (73%)	4	4

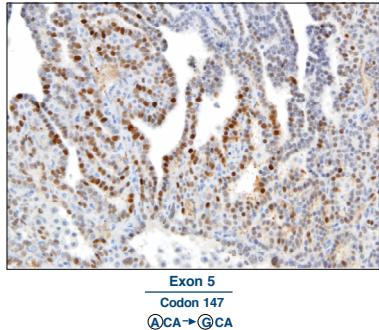
\*Male Swiss (CD-1) mice were exposed to 0, 50, 100, 200, or 300 mg/kg AZT

Predominant p53 Mutations in Lung Neoplasms from Swiss (CD-1)  
Male Mice in the AZT Transplacental Carcinogenesis Study

Exon 5  
Codon 147  
~~A~~CA → GCA  
Thr → Ala

Exon 8  
Codon 285  
~~A~~AT → AT  
Asn → Ile

**p53 Protein Expression in Alveolar Bronchiolar Carcinoma from Swiss (CD-1) Male Mice in the AZT Transplacental Carcinogenesis Study**



Exon 5  
Codon 147  
ⒶCA→ⒼCA

**NIEHS analysis of AZT levels in CD-1 Dam and fetus**

B. Collins (NIEHS) and E. Garner (RTI)  
Preliminary Results



**Study Design**

- ◆ Pregnant CD-1 Mice dosed with AZT on gestational day 10 - 17 (oral gavage)
- ◆ Parent AZT levels measured on GD 17
- ◆ Maternal plasma level
- ◆ Fetal Tissue level
- ◆ Total AZT estimated (parent ~ 80% of total AZT)

